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(57) Abstract

Use of an amphiphilic compound in the manufacture of a medicament for the inhibition of phosphatidylcholine synthesis, said amphiphilic compound have the following properties: i) the compound copmprises a non-ionic, cationic or anionic hydrophilic head group and a hydrophobic tail group; ii) the head group has a cross section A and the tail group has a cross section B such that the ratio B:A is less than 0.7:1; iii) the tail group comprises a straight hydrocarbon chain having from 8 to 18 carbon atoms; and iv) the amphiphilic compound has a membrane/water partition coefficient of more than 1×10^{-3}.

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PHOSPHATIDYLCHOLINE SYNTHESIS INHIBITORS

The present application relates to compounds which act as inhibitors of phosphatidylcholine synthesis, and which can be used in, for example, the treatment of cancers.

The use of amphiphilic compounds as anti-neoplastic agents is attractive because they represent a class of non-DNA interactive therapeutic agents and may therefore be non-serotoxic. *In vitro* laboratory studies have shown that certain amphiphilic compounds, such as the phospholipid analogues Edelfosine and Mitelfosine (trade marks) are able to induce cytostasis in cancer cell cultures at micromolar concentrations. It has also been shown that at these concentrations the non-cancerous cells are relatively unaffected by these compounds.

The activity of these compounds depends on the ability of the molecules to insert into cell membranes. Insertion into membranes affects the activity of several membrane-associated proteins, in particular protein kinase C (PKC) and CTP:phosphocholine cytidylyltransferase (CT). CT controls the rate limiting step in the synthesis of phosphatidylcholine (PC) lipids. By inhibiting CT cells are prevented from increasing their lipid mass and are unable to divide. Phospholipid analogues decrease the activity of CT thereby suppressing PC synthesis. Inhibition of PC synthesis affects DNA synthesis. As a consequence cells exposed to these compounds are unable to divide and subsequently die. Clinical trials of Mitelfosine have shown it to be of particular benefit in the treatment of breast cancer skin metastasis and cutaneous lymphomas.

We have found that the activity of amphiphilic compounds depends on a number of factors. We have found that the inhibition of growth in cancer cell cultures is correlated with the ratio of the cross-section of the head group to the cross section of its hydrophobic parts. Compounds with large head groups have higher activity than compounds with small head groups. Compounds with large head groups induce strong geometric and elastic perturbations in bilayer membranes. The extent of these perturbations correlates with their ability to affect the activity of CT and PKC and other membrane-associated proteins (eg. adenylate cyclase, phosphatididate phosphohydrolase, diacylglycerol kinase, DAG: choline phosphotransferase, phospholipase C and phospholipase A_2). Furthermore it has been found that the activity depends on the chemical nature of the head group. Activity generally increases along the series zwitterionic < anionic < nonionic < cationic. Edelfosine and Mitelfosine both have zwitterionic head groups. For anionic amphiphilic compounds the salts are less active than the protonated compounds. In amphiphilic compounds that contain one or two hydrocarbon chains it has been found that the activity depends on the length of the hydrocarbon chains

The present invention relates to compounds which inhibit phosphatidylcholine synthesis and which can be used, for example, in the treatment of cancers. The compounds may have higher activity than known amphiphilic compounds such as edelfosine and mitelfosine.

The present invention provides the use of an amphiphilic compound in the manufacture of a medicament for the inhibition of phosphatidylcholine

synthesis, said amphiphilic compound have the following properties:

i) the compound comprises a non-ionic, cationic or anionic hydrophilic head group and a hydrophobic tail group,

ii) the head group has a cross section A and the tail group has a cross section B such that the ratio B:A is less than 0.7:1,

iii) the tail group comprises a straight hydrocarbon chain having from 8 to 18 carbon atoms, and

iv) the amphiphilic compound has a membrane/water partition coefficient of more than 1×10^{-3}.

In the present invention an amphiphilic compound is to be understood as being a compound having a hydrophilic head attached to a hydrophobic tail, although there may be more than one hydrophobic tail. For example, the heads of two amphiphilic compounds each having a single head and a single tail may be joined either directly or via a short linking group such as an alkylene group having from 1 to 6, preferable 1 to 4, carbon atoms which does not materially affect the hydrophilicity of the head groups.

Amphiphilic compounds having a cationic head group have generally been found to have the best phosphatidylcholine synthesis inhibition activity. However, amphiphilic compounds having a non-ionic head group may have better properties in other areas. For example, they may have less haemolytic activity *in vivo*.

The ratio B:A is preferably as small as possible. For example it may be less than 0.5:1, preferably less

than 0.3:1. From a synthetic viewpoint, it is difficult to obtain compounds having a ratio B:A of less than 0.25:1. Amphiphilic compounds having this characteristic are sometimes referred to as "type I amphiphiles" because they form normal topology liquid crystalline phases when mixed with water.

The absolute values for A and B do not matter; it is the ratio which is important. Values for A and B may be obtained from measurements on space-filling molecular models, from the use of empirical formulae using the method disclosed in J. Isrealachvili (1982), "Intermolecular & Surface Forces", Academic Press, London, or from studies of X-ray diffraction data obtained from the liquid crystalline phases formed by these materials using the method disclosed in J. M. Seddon (1990), Biochimica et Biophysica Acta, vol. 1031, pp1-69.

The tail group is a straight chain hydrocarbon group containing from 8 to 18 carbon atoms. It is preferably an alkyl group, although it may contain one or more carbon-carbon double bonds. In order to ensure that the tail has a small cross section, these bonds must be in the trans configuration. The tail preferably contains from 12 to 16 carbon atoms, and may contain, for example, 13, 14 or 15 carbon atoms, and none, one or two carbon-carbon double bonds.

The membrane/water partition coefficient of the amphiphilic compound is more than 1×10^{-3}, and is preferably as large as possible, ie. preferably more than 5×10^{-3}, more preferably more than 1×10^{-2}, even more preferably more than 1×10^{-1}. This coefficient is the ratio of the concentration of the compound in the membrane/concentration of the compound in the

solution. The coefficients may be measured in accordance with the method of E.E. Kelley, E.J. Modest, C.P. Burns (1993), Biochemical Pharmacology, vol. 45, pp 435-2439.

Examples of amphiphilic compounds which can be used in the present invention are as follows:

An oligoethyleneglycol monoalkyl ether of formula (I):

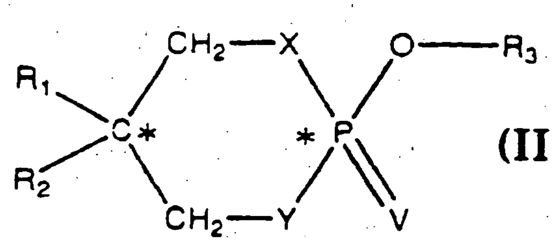
$$R_1—(OCH_2CH_2)_n\text{-}OH \qquad \text{(I)}$$

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms, and

n is from 6 to 12, preferably 8 to 10.

An alkyl malonyl phosphoanhydride of formula (II):



(II)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing up to 5 carbon atoms,

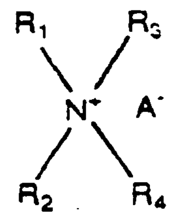
R_3 is hydrogen, a monovalent cation [insert examples] or a choline group,

X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH, and

V is oxygen or sulfur.

An alkylammonium compound of formula (III):



(III)

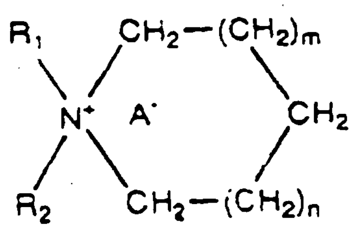
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2, R_3 and R_4, which may be identical or different, are each a methyl, ethyl or straight or branched propyl group, and

A^- is a pharmaceutically acceptable anion.

An alkylammonium compound of formula (IV):



(IV)

wherein:

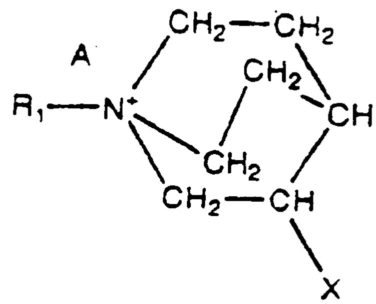
R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is methyl,

m and n are integers such that the ring contains from 5 to 8 ring atoms, preferably 6 ring atoms, and

A^- is a pharmaceutically acceptable anion.

An alkylammonium compound of formula (V):



(V)

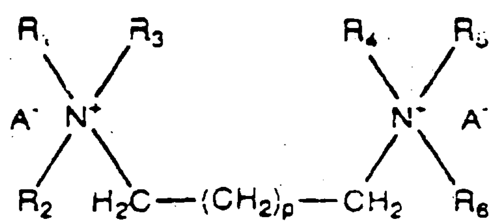
wherein:

R_1 is a straight hydrocarbon group containing from 8 to 18 carbon atoms,

X is hydrogen, and

A^- is a pharmaceutically acceptable anion.

An alkylammonium compound of formula (VI):



(VI)

wherein:

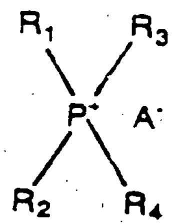
R_1, R_3, R_4 and R_5, which may be identical or different, are each methyl or ethyl,

R_2 and R_6, which may be identical or different, are each straight hydrocarbon groups containing from 10 to 18 carbon atoms

p is from 2 to 4, preferably 3, and

each A^- which may be identical or different, is a pharmaceutically acceptable anion.

An alkylphosphonium compound of formula (VIII):



(VIII)

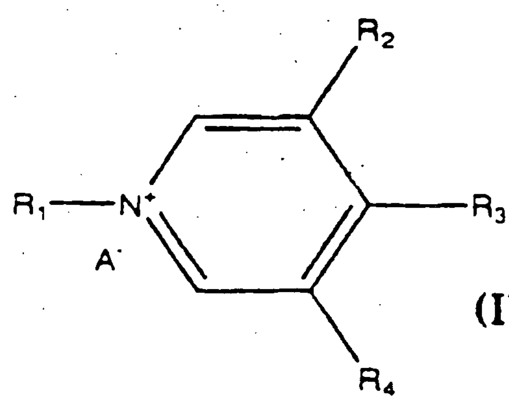
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2, R_3 and R_4, which may be the same or different, are each methyl, ethyl or straight or branched propyl groups, and

A^- is a pharmaceutically acceptable anion.

An alkylpyridinium compound of formula (IX):



(IX)

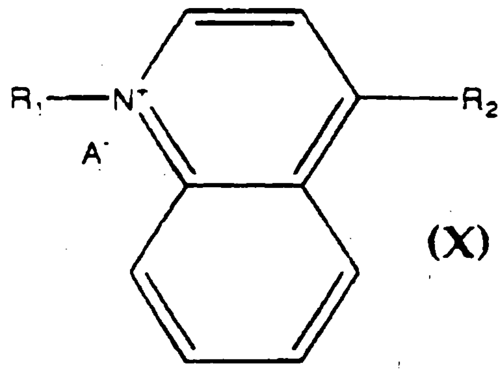
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2, R_3 and R_4 are each hydrogen, and

A^- is a pharmaceutically acceptable anion.

An alkylpyridinium compound of formula (X):



(X)

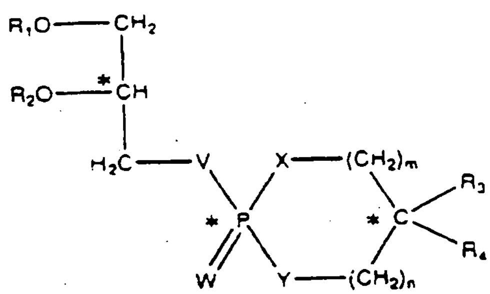
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen, and

A^- is a pharmaceutically acceptable anion.

An alkyl trisubstituted phosphate of formula (XI):



(XI)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing not more than 5 carbon atoms,

R_3 and R_4, which may be identical or different, are each a straight or branched alkyl group containing not more than 5 carbon atoms,

m and n are integers such that the ring contains from 5 to 8 ring atoms, preferably 6 ring atoms,

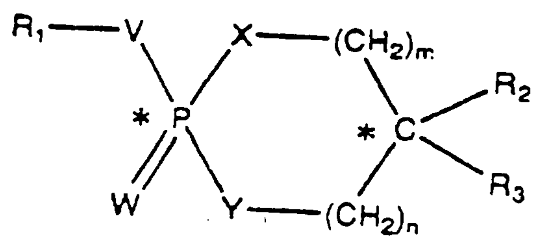
X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH

V is oxygen or sulfur, and

W is oxygen or sulfur.

An alkyl trisubstituted phosphate of formula (XII):



(XII)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is a straight or branched alkyl group containing not more than 5 carbon atoms,

R_3 is a straight or branched alkyl group containing not more than 5 carbon atoms,

m and n are integers such that the ring contains from 5 to 8 ring atoms, preferably 6 ring atoms,

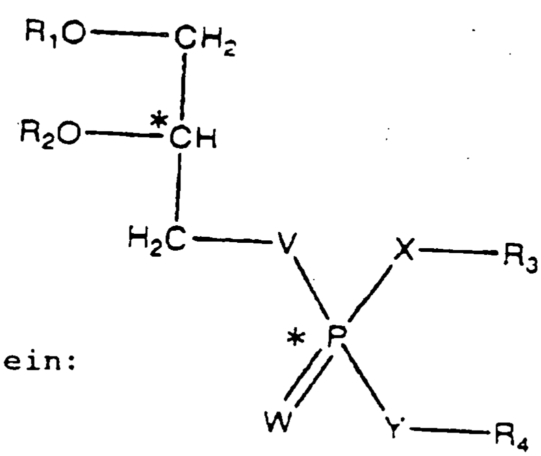
X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH

V is oxygen or sulfur, and

W is oxygen or sulfur.

A trisubstituted phosphate of formula (XIII):



(XIII)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing not more than 5 carbon atoms,

R_3 and R_4, which may be identical or different, are each a straight or branched alkyl group containing not more than 5 carbon atoms,

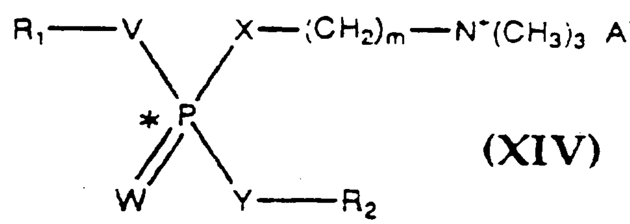
X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH

V is oxygen or sulfur, and

W is oxygen or sulfur.

A trisubstituted phosphate of formula (XIV):



(XIV)

wherein:

R_1 is a hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing not more than 5 carbon atoms,

m is from 2 to 6, preferably 3 to 5, most preferably 4,

X is oxygen, sulfur or NH,

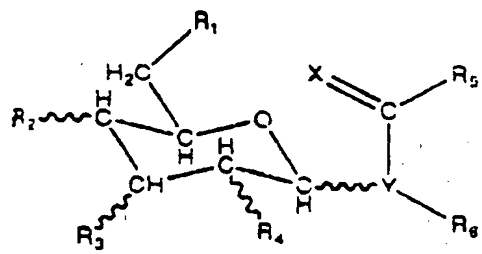
Y is oxygen, sulfur or NH

V is oxygen or sulfur,

W is oxygen or sulfur, and

A^- is a pharmaceutically acceptable anion.

A glycolipid analogue of formula (XV):



(XV)

wherein:

R_1 is an amino, trimethyl ammonium, triethyl ammonium or phosphate group,

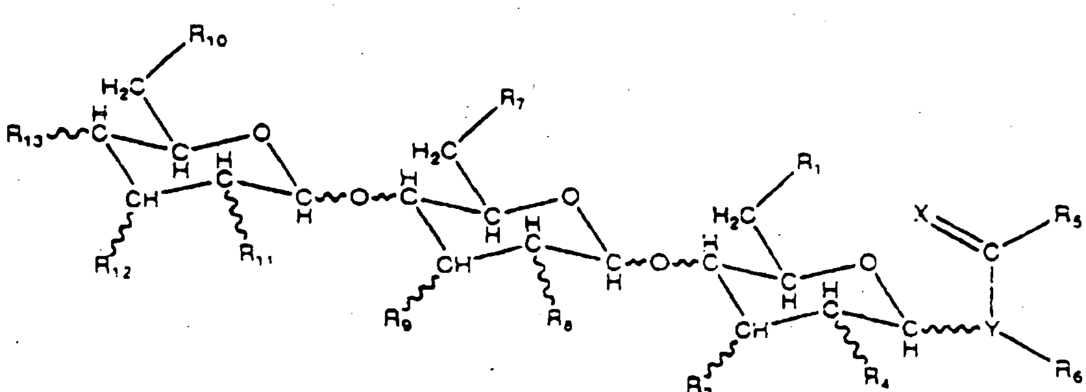
R_2 is a hydroxy or phosphate group,

R_3 is a hydroxy or phosphate group,

R_4 is hydrogen, and

one of R_5 and R_6 is methyl, ethyl or straight or branched propyl and the other is a straight hydrocarbon group containing from 8 to 18 carbon atoms.

A glycolipid analogue of formula (XVI):



(XVI)

wherein:

R_1, R_7 and R_{10}, which may be identical or different, is each an amino, trimethyl ammonium, triethyl ammonium or phosphate group,

R_{13} is a hydroxy or phosphate group,

R_3, R_9 and R_{12}, which may be identical or different, is each a hydroxy or phosphate group,

R_4, R_8 and R_{11} is each hydrogen, and

one of R_5 and R_6 is methyl, ethyl or straight or branched propyl and the other is a straight hydrocarbon group containing from 8 to 18 carbon atoms.

If R_1, R_7 and R_{10} in formulae (XV) and (XVI) is a trimethyl ammonium or a triethyl ammonium group, it contains a balancing anion. This anion may be an anion A^- as herein defined.

The pharmaceutically acceptable anion A^- may be inorganic or organic. Examples of inorganic anions are halides such as fluoride, chloride and bromide. Examples of organic anions are methylsulfate, toluenesulfate and acetate.

The straight hydrocarbon groups are generally alkyl groups. They may optionally contain one, two or more trans carbon-carbon double bonds. The groups containing from 10 to 18 carbon atoms or 8 to 18 carbon atoms preferably contain from 12 to 18 carbon atoms, more preferably from 14 to 18 carbon atoms, most preferably 16 carbon atoms. The hydrocarbon group containing from 8 to 18 carbon atoms in the compound of formula (V) preferably has from 10 to 16 carbon atoms, more preferably from 10 to 14 carbon atoms, most preferably 12 carbon atoms.

The alkyl groups containing from 1 to 5 carbon atoms may be straight or branched. Examples are methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl and n-pentyl.

The present invention also provides a method of inhibiting phosphatidylcholine synthesis in a subject, which comprises administering to a subject in need of such inhibition an effective amount of an amphiphilic compound as herein defined.

The amphiphilic compounds used in the present invention may be used, for example, to treat a cancer. The compounds may be administered, for example, by intravenous (i.v.) injection or topically. They may also be used in autologous bone marrow transplantation.

The compounds may be administered in any conventional form. Thus they may be administered in the form of a pharmaceutical composition comprising the compound and a pharmaceutically acceptable carrier or diluent. The compounds may be used singly or in a combination of two or more. The compounds may also be administered either together or separately with other compounds useful in the treatment of cancers.

The amphiphilic compounds may be used to treat a variety of cancers. They may be used, for example, for the treatment of leukemias, breast cancer skin metastasis, cutaneous lymphomas, mammary carcinomas, fibrosarcomas, melanomas and lung carcinomas. They may also be used as purging agents for autologous bone marrow transplantation.

The amphiphilic compounds may be administered, for example, to humans by injection at a dose of 5 to 15 mg/kg body weight per injection, or by topical application in an amount of not less than 50, preferably 80, mg/kg body weight. They may also be used in an amount of not less than 3, preferably 10, more preferably 20, micromoles per 10^6 lymphocytes for bone marrow purging.

Some of the amphiphilic compounds used in the present invention can be obtained from commercial sources, although they may require further purification, for example by column chromatography or by recrystallization before use.

Compounds of formula (I) may be obtained from Fluka Chemie AG.

Compounds of formula (III) may be purchased from Aldrich Chemical Co.

Compounds of formula (IV) may be synthesized by the alkylation of an N-methyl heterocycle with the appropriate 1-bromoalkane. An example of a synthesis method is given in Example 10. Other compounds of formula (IV) may be prepared by analogous methods.

Compounds of formula (V) may be synthesised by the alkylation of quinuclidene or quinuclidinol with the appropriate 1-bromoalkane. An example of a synthesis method is given in Example 10. Other compounds of formula (V) may be prepared by analogous methods.

Compounds of formula (VI) may be synthesised by the alkylation of N,N,N′,N′ tetraalkyl (alkylene diamine) with the appropriate 1-bromoalkane. An example of a

synthesis method is given in Example 10. Other compounds of formula (VI) may be prepared by analogous methods.

Compounds of formula (VIII) may be synthesised by the alkylation of trialkyl phosphine with the appropriate 1-bromolkane. An example of a synthesis method is given in Example 10. Other compounds of formula (VIII) may be prepared by analogous methods.

Compounds of formula (IX) may be synthesised by the alkylation of pyridine or a pyridine derivative with the appropriate 1-bromoalkane. An example of a synthesis method is given in Example 10. Other compounds of formula (IX) may be prepared by analogous methods.

Compounds of formula (X) may be synthesised by the alkylation of quinoline with the appropriate 1-bromoalkane. An example of a synthesis method is given in Example 10. Other compounds of formula (X) may be prepared by analogous methods.

Some of the amphiphilic compounds used in the present invention are novel. These include the compounds of formulae (II),(XI),(XII),(XIV),(XV)and(XVI).

The compounds of formula (II) may be prepared by alkylation of diethylmalonate using the appropriate 1-bromoalkanes. An example of a synthesis method is given in Example 2. Other compounds of formula (II) can be prepared by analogous methods.

The present invention also provides an amphiphilic compound as defined above for use in a method of treatment of the human or animal body by therapy.

The present invention is now further described in the following Examples.

In the following Examples the activities given are the reciprocals of the concentrations required to produce 50% mortality (EC_{50}) in *in vitro* assays, relative to a control group to which no agent is added.

HL60, Ramos and Daudi cancer cell lines were cultivated in RPMI 1640 medium supplemented with 10% foetal bovine serum and 2 millimolar glutamine. The cells were maintained in a humidified atmosphere with 5% carbon dioxide at 37 degrees Celsius. Cell viability as a function of different concentrations of amphiphilic compounds was determined using a colorimetric assay. Cells were seeded at 4 x 10^5 cells per millilitre for HL60 cells, 8 x 10^5 per millilitre for Daudi cells and 2 x 10^5 cells per millilitre for Ramos cells. All of the amphiphilic compounds were made up as 11 millimolar stock solutions in medium. These solutions were further diluted such that the final concentrations used in the assays were 0, 0.1, 0.5, 1, 5, 10, 50 and 100 micromolar. Cells were added to the culture plates in a volume of 100 micromolar. The amphiphilic compounds were added in 10 micromolar aliquots. Cultures were incubated at 37 degrees Celsius. After this incubation 10 microlitres of a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in buffer were added to the cultures and the cells were incubated for a further four hours. 100 microlitres of acidified isopropanol were added to dissolve the formazan product. The optical density was measured at 560 nanometers.

Example 1

A number of compounds of formula (I) as defined above were purchased from Fluka Chemie AG and purified by column chromatography using silica as the stationary phase and petroleum ether/chloroform (7:3) as the mobile phase. In these compounds $R_1 = CH_3(CH_2)_{m-1}$ wherein m=10,12,14,16 or 18 and n=8.

The activities of these compounds were assayed against HL60, Ramos and Daudi cells. The results are shown in Table 1.

Table 1

m	EC_{50} (HL60)/μM	EC_{50}(Ramos)/μM	EC_{50}(Daudi)/μM
10	18.8	4.9	2.3
12	8.4	1.0	0.7
14	6.2	0.5	0.4
16	5.1	0.4	0.4
18	3.1	0.3	0.2

Example 2

A number of compounds of formula (II) were synthesised by alkylation of diethyl malonate using the appropriate 1-bromoalkanes according to literature procedures. The alkyl diethylmalonate was reduced to produce the diol which was phosphorylated using phosphorus oxychloride. Salts were prepared by reacting the product with, for example, sodium ethoxide. The compounds prepared were those of formula (II) in which $R_1=CH_3(CH_2)_{m-1}$ and R_2=H, R_3=H, m=12,14,16 or 18 and X=Y=V=O.

The method of synthesis was as follows. This method prepares a compound wherein m=12. Other compounds of formula (II) may be prepared by analogous methods.

All reactions requiring dry conditions were performed under nitrogen, using dry solvents. Dichloromethane, ether, chloroform, and triethylamine were distilled over calcium anhydride and stored over 4A sieves before use. Ethanol was dried by distillation over magnesium turnings and iodine. Phosphorus oxychloride was distilled and stored under nitrogen. Reactions were monitored by thin layer chromotography (TLC), employing precoated Alugram SIL G/UV254 plates. TLC plates were developed either with 5% sulphuric acid in ethanol, 5% phosphomolybdic acid in methanol, or iodine on silica gel. Flash chromotography was carried out using silica gel.

Synthesis of diethyl dodecylmalonate (reaction intermediate A).

A solution of sodium ethoxide was prepared by carefully dissolving sodium metal (1.85g, 0.08 mol) in dry ethanol (20 ml). Diethyl malonate (13.28g, 0.083 mol) was introduced dropwise, followed by the addition of 1-bromododecane (20g, 0.08mol). The mixture was refluxed under dry N_2 overnight. Ethanol was removed from the mixture by rotary evaporator, and dichloromethane (100ml) was used to redissolve the residue. The mixture was washed with water (100ml), then dried with $MgSO_4$, and concentrated to given an orange oil (23.56g). Some of the residual diethyl malonate was removed by short path distillation. The remaining crude product was purified by column chromatography (1:1 ethyl acetate/petroleum ether) to

yield the product (A) as a colourless solid (20.27g, 74% yield).

Synthesis of 2-dodecyl-1,3-propanediol (reaction intermediate B).

Lithium aluminium hydride (2.17g, 0.57mol) was carefully introduced into a dry flask under N_2 and mixed thoroughly with dry ether (200ml). Diethyl dodecylmalonate (A) (17.93g, 0.55mol) was cautiously added to the lithium aluminium hydride mixture with vigorous stirring. Traces of the added ester were washed into the reaction vessel with dry ether. Further portions of dry ether (4x50ml) were added to facilitate stirring as the mixture became more viscous. After 3 hours of stirring, the excess aluminium hydride was decomposed by the controlled addition of water (100ml). Chloroform (200ml) was added and the aqueous layer was separated. The organic extract was dried with $MgSO_4$ and concentrated. The residual solid was recrystallised from methanol to yield the desired product (B) as a white solid (9.55g, 71% yield).

Synthesis of 5-dodecyl-2-chloro-1,3-dioxa-2-phosphacyclohexane-2-oxide (reaction intermediate C)

Phosphorus oxychloride (0.28g, 1.84mmol, 1.5eq) in dry dichloromethane (100ml) was introduced dropwise into a solution of 2-dodecyl-1,3-propanediol (B) (0.30g, 1.23mmol 1eq) in dichloromethane (100ml) containing triethylamine (1.24g, 12.3mmol, 10eq) with stirring under N_2 at room temperature. After 3h, the solved and remaining volatile resides were removed under high vacuum. The resulting solid was chromatography (R_f 0.50 & 0.60 1:1 ethyl acetate/petroleum ether) to

yield the pure product 5-dodecyl-2-chloro-1,3-dioxa-2-phosphacyclohexane-2-oxide (C) (0.40g, 100% yield) as two diastereomers.

Synthesis of 5-dodecyl-2-hydroxy-1,3-dioxa-2-phosphacyclohexane-2-oxide (target compound I, $R_1=CH_3(CH_2)_{m-1}$, $R_2=H$, $R_3=H$, m=12 and X=Y=V=O). Water (30ml) was added to a solution of 5-dodecyl-2-chloro-1,3-dioxa-2-phosphacyclohexane-2-oxide (C) (1.00g, 3.08mmol) in acetonitrile (50ml) at room temperature. The mixture was allowed to stir at room temperature overnight, and was observed to become cloudy. A 2:1 mixture of chloroform/methanol (25ml) was added, and the resulting phases were separated. The aqueous phase was extracted with 2:1 mixture of chloroform/water (2x30ml). Water (3x30ml), that had been acidified to pH 3 with dilute hydrochloric acid, was used to wash the organic layer. The organic extracts were then combined and dried over $MgSO_4$. Concentration of the extract on a rotary evaporator, followed by freeze-drying yielded the pure product (0.97g, 100%).

The activities of these compounds were assayed against HL60, Ramos and Daudi cells. The results are shown in Table 2

Table 2

m	EC_{50} (HL60)/μM	EC_{50}(Ramos)/μM	EC_{50}(Daudi)/μM
12	391.0	92.9	88.4
14	103.7	23.0	37.3
16	63.6	18.1	23.8
18	20.8	8.4	16.5

Example 3

A number of compounds of formula (III) were purchased from Aldrich Chemical Co. and were purified by a three-fold recrystallization from absolute ethanol. The compounds prepared were those of formula (III) in which $R_1 = CH_3(CH_2)_{m-1}$, $R_2=R_3=R_4=$ methyl, A^-=bromide and m=12,14,16 or 18.

The activities of these compounds were assayed against HL60, Ramos and Daudi cells. The results are shown in Table 3

Table 3

m	EC_{50} (HL60)/μM	EC_{50}(Ramos)/μM	EC_{50}(Daudi)/μM
12	3.7	0.3	1.6
14	2.1	0.2	1.3
16	1.4	0.1	0.9
18	1.0	0.08	0.8

Example 4

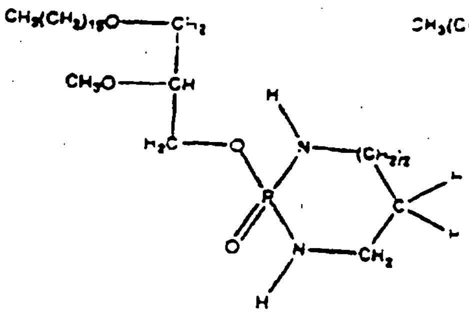
A number of compounds of formulae (XI), (XII), (XIII) and (XIV) were synthesised. Compounds of formula (XI) were synthesised from *rac* solketal to produce a 3-O-alkyl-2-O-methyl-*rac*-glycerol according to procedures described in the literature. Protection of the hydroxy group at position 1 allowed the alkylation of the hydroxy at position 2. These intermediates were reacted with phosphorus oxychlodire to produce phosphorodichloridate intermediates by procedures described in the literature. These were reacted with alpha,omega-difunctional amines. Alcohols or amino alcohols to produce the desired cyclic compounds.

Separation of the isomers was achieved using literature procedures.

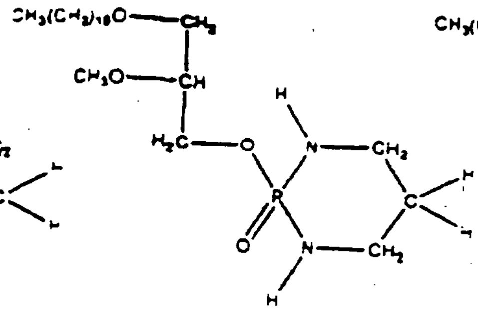
The compounds of formula (XII) were obtained in a similar manner using appropriate n-alkanols as starting materials. Compounds of formulae (XIII) were obtained by reacting the alkylglycerol intermediates with the appropriate phosphorochloridate followed by treatment with trimethylamine. Compounds of formulae (XIV) were obtained in a similar manner using the appropriate n-alkanol as starting materials.

The activities of a number of compounds were assayed over a range of cell lines. Representative EC_{50} data are given in Table 4.

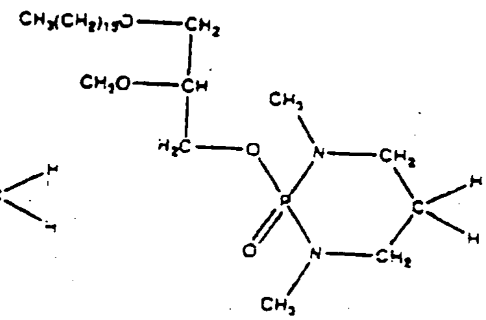
Table 4



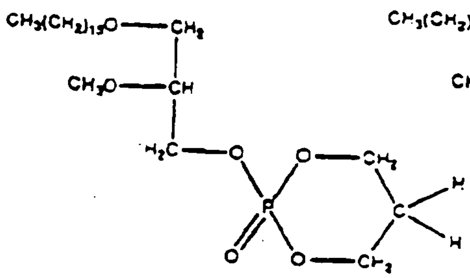
cell line	EC_{50} (μM)
HL60	3
CEM	12
Daudi	10
Ramos	3



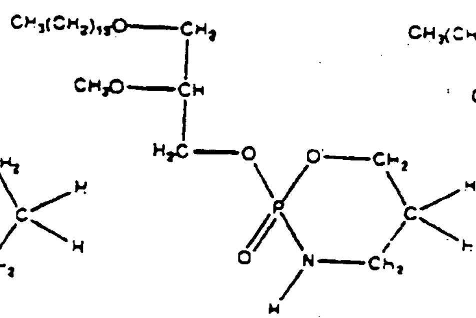
cell line	EC_{50} (μM)
HL60	8



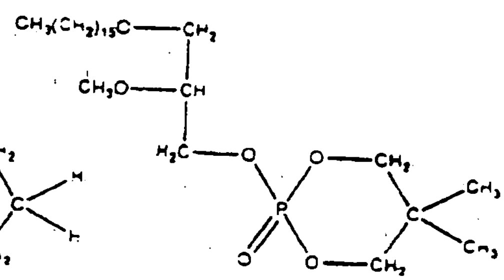
cell line	EC_{50} (μM)
HL60	15



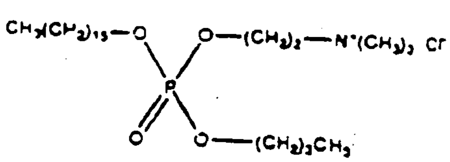
cell line	EC_{50} (μM)
HL60	15
CEM	40
Daudi	28
Ramos	12



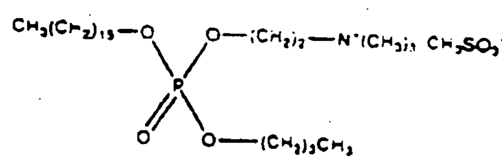
cell line	EC_{50} (μM)
HL60	30



cell line	EC_{50} (μM)
HL60	7



cell line	EC_{50} (μM)
HL60	3
CEM	30
Daudi	15
Ramos	5



cell line	EC_{50} (μM)
HL60	9

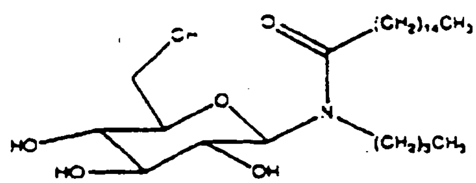
TABLE 4

Example 5

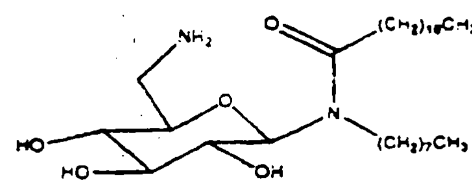
A number of compounds of formulae (XV) and (XVI) were synthesised by the reaction of the appropriate carbohydrate and alkylamine followed by acylation by procedures described in the literature. Selective functionalization of the carbohydrate hydroxy groups was acheived using benzyl protection group chemistry according to literature procedures.

The activities of a number of compounds were assayed against HL60 cells. Representative EC_{50} data are given in Table 5

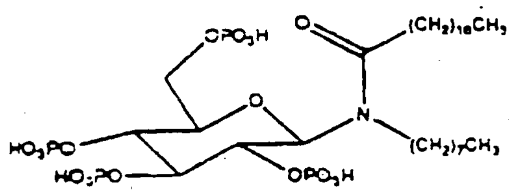
Table 5



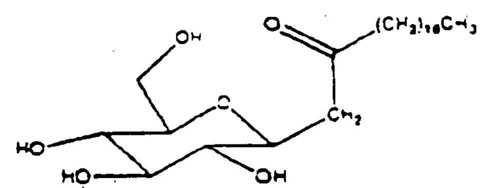
EC$_{50}$ (µM)/HL60 : 21



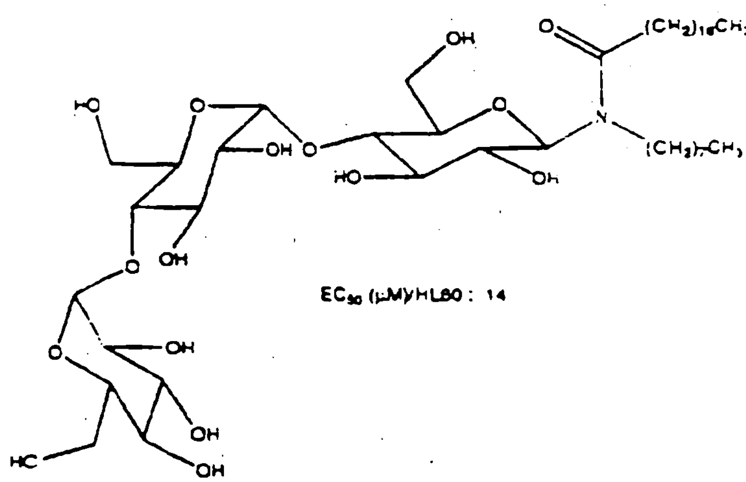
EC$_{50}$ (µM)/HL60 : 9



EC$_{50}$ (µM)/HL60 : 20



EC$_{50}$ (µM)/HL60 : 8



EC$_{50}$ (µM)/HL60 : 14

TABLE 5

Example 6

The haemolytic activity of a number of amphiphilic compounds was determined This was expressed as HC_{50} values, which was the concentration that produced 50% haemolysis as determined by a standard colorimetric assay following 2 minutes incubation with fresh mouse red blood cells. The HC_{50} data are given below:

Compound	HC_{50} (millimoles)
Compound of formula (I) in which n=8 and m=14	0.128
Compound of formula (I) in which n=8 and m=16	0.173
Compound of formula (III) in which R_2=R_3=R_4=methyl, R_1=C_{14} alkyl and A=Br	0.199
Compound of formula (III) in which R_1=R_2=R_4=methyl, R_1=C_{16} alkyl and A=Br	0.093
Compound of formula (XIV) in which R_1=C_{16} alkyl, m=2 W=X=Y=O, R_2=butyl and A=Cl	0.113
Mitelfosine	0.044

These data show that the amphiphilic compounds used in the present invention induce less haemolysis than mitelfosine. They may therefore, if desired, be administered i.v. at higher concentrations than Mitelfosine.

Example 7

The inhibition of cell cycle in HL60 human promyelocytic leukemia cells was investigated by flow cypometry using bromodeoxyuridine as a fluorescent stain. The results obtained are given in Table 6, which shows the percentage of the cells in each phase.

Table 6

Compound	Phase of cell cycle		
	G1	S	G2/M
Control	55.1(± 0.5)	36.7(± 1.5)	8.3(±1.7)
Mitelfosine	54.9(± 0.7)	37.3(± 1.8)	7.8(± 1.2)
(I)	45.8(±2.8)	53.6(±3.1)	0.7(±0.1)
(III)	45.0(±2.1)	46.8(±1.7)	8.1(±0.5)
(XIV)	61.1(±0.9)	31.1(±1.3)	7.8(±1.7)

Mitelfosine was tested at 3 micromolar for 72 hours.

The compound of formula (I) was one in which n=8 and m=16, and was tested at 0.8 micromolar for 72 hours.

The compound of formula (III) was one in which $R_2=R_3=R_4$=methyl, $R_1=C_{16}$ alkyl and A=Br, and was tested at 1 micromolar for 72 hours.

The compound of formula (XIV) was one in which $R_1=C_{16}$ alkyl, W=X=Y=O, R_2=butyl and A=Cl and was tested at 2 micromolar for 72 hours.

These data show that there is a different pattern of activity between mitelfosine and the amphiphilic

compounds used in the present invention. Mitelfosine does not appear to change the proportions of the cells in the different phases of the cell cycle. The non-ionic compound (I) and the cationic compound (III) appear to block cells in the S phase. The cationic compound (XIV) appears to block cells in the G1 phase.

Example 8

The effect of amphiphilic compounds on phosphatidylcholine (PC) and phosphatidylethanolamine (PE) concentrations in membranes of HL60 cells was determined.

Data were obtained using electrospray ionization mass spectrometry from total lipid extract of HL60 cells. Following treatment with the test compounds, cell suspensions were centrifuged and washed with ice-cold buffer. 1.5 nanomoles dimyristoyl PC and 4.0 nanomoles dimyrisoyl PE were added as internal standards. Lipids were extracted using the method of Bligh and Dyer (E. G. Bligh and W. S. Dyer (1959), Canadian Journal of Biochemistry and Physiology, vol. 37, pp911-923). The PC and PE fractions were separated using aminopropyl Bondelut columns. Samples for mass spectrometry were dissolved in 5 millimolar sodium hydroxide dissolved in chloroform/methanol (2:1). The mobile phase employed consisted of methanol/chloroform/water (80:10:10). The PE lipids were quantitated using the negative ionization mode while the PC lipids were quantitated as their sodium adducts using the positive ionization mode.

The results are given in Table 7.

Table 7

C mpound	Concentration of PC in nanomoles per 10^7 cells	Concentration of PE in nanomoles per 10^7 cells
Control	138	128
Mitelfosine	118	146
(I)	106	168
(III)	110	161

The compounds (I) and (III) and the treatment concentrations and times for these compounds and the Mitelfosine are the same as those set out in Example 7.

These data show that the treatment of cells with amphiphilic compounds used in the present invention leads to a reduction in the amount of PC lipids and an increase in the amount of PE lipids. These observations are consistent with these compounds inhibiting the activity of the enzyme CT. The compounds are more efficient at decreasing PC than mitelfosine.

Example 9

The reduction in PC synthesis of various compounds used in the present application was determined.

Incorporation of radioactively-labelled choline into the total phosphatidylcholine-containing lipid fraction of the HL60 cells was measured after exposure over 48 hours to the following compounds at the following concentrations.

Metelfosine	5 micromolar
Compound of formula (I) in which $R_1=CH_3(CH_2)_{15}$ and n=8	1 micromolar
Compound formula (III) in which $R_1=R_2=R_3=$methyl, $R_4=CH_3\ (CH_2)_{15}$ and A=Br	3 micromolar

The results obtained are shown in Table 8.

Table 8

Compound	Incorporation of ^{14}C choline into phosphatidylcholine (% of control)
Control	100
Mitelfosine	48(±8)
(I)	54(±11)
(III)	46(±12)

The reduction in the rate of PC synthesis indicates that the amphiphilic compounds used in the present invention are potent inhibitors of the enzyme CTP: phophocholine cytidylyltransferase (CT).

Example 10

A number of amphiphilic compounds which are used in the present invention were synthesised.

Compounds of formula (IV).

A number of compounds of formula (IV) as defined above were synthesised by the alkylation of a N-methyl heterocycles with the appropriate 1-bromoalkanes. The compounds prepared were those of formula (IV) in which $R_1=CH_3(CH_2)_{m-1}$ wherein m'=12, 14, 16, 18, R_2=methyl, m=n=1 and A-=Cl^- or Br^-. The method of preparation is illustrated by the synthesis of the homologue with m'=16 and $A^-=Br^-$.

Synthesis of N-hexadecyl-(N-methyl)-piperidinium bromide.

Equimolar quantities (5mmol) of N-methyl piperidine (0.5g) and 1-bromohexadecane (1.5ml) were dissolved in absolute ethanol (20ml) and refluxed under nitrogen for 48 hours with rigorous exclusion of moisture. The precipitate obtained from this reaction was recrystallised 3 times from dried methanol to afford the target compound as a white solid (1.71g, 85% yield).

Compounds of formula (V).

A number of compounds of formula (V) as defined above were synthesised by the alkylation of quinuclidene or quinuclidinol with the appropriate 1-bromoalkanes. The compounds prepared were those of formula (V) in which $R_1=CH_3(CH_2)m_{-1}$ wherein m=8, 10, 12, 14, 16, 18, X=H and $A^-=Cl^-$ or Br^-. The method of preparation is illustrated by the synthesis of the homologue with m=16 and $A^-=Br^-$.

Synthesis of N-hexadecyl-1-azoniabicyclo [2.2.2.] octane bromide.

Equimolar quantities (5mmol) of 1-bromohexadecane (1.5ml) and quinuclidene (0.56g) were dissolved in dried methanol (20ml) and refluxed under nitrogen for 10 hours with rigorous exclusion of moisture. The cooled reaction mixture was poured into 300 ml of diethyl ether and the resulting precipitate was collected by vacuum filtration. The product was obtained as a white solid following 4 recrystallizations from absolute ethanol (1.3g, 65% yield).

Compounds of Formula (VI).

A number of compounds of formula (VI) as defined above were synthesised by the alkylation of (N,N,N′,N′-tetraalkyl (alkane diamine) with the appropriate 1-bromoalkanes. The compounds prepared were those of formula (VI) in which $R_2=R_6=CH_3(CH_2)_{m-1}$ wherein m=12, 14, 16, 18, $R_1=R_3=R_4=R_5$=methyl, p=4 and $A^-=Br^-$. The method of preparation is illustrated by the synthesis of the homologue with m=16.

Synthesis of N, N′-dihexadecyl- (N N N′,N′-tetramethyl) hexanediammonium dibromide..

1-bromohexadecane (7.8mmol, 2.4ml) and (N, N, N'N'-tetramethyl hexane diamine (2.6mmol, 0.56ml) were dissolved in dried methanol (20ml) and refluxed under nitrogen for 10 hours with rigorous exclusion of moisture. The cooled reaction mixture was poured into 300 ml of diethyl ether and the resulting precipitate was collected by a vacuum filtration. The product was obtained as a white solid following 4 recrystallizations from absolute ethanol (1.33g, 67% yield).

Compounds of formula (VIII).

A number of compounds of formula (VIII) as defined above were synthesised by the alkylation of trialkylphosphine with the appropriate 1-bromoalkanes. The compounds prepared were those of formula (VIII) in which $R_1=CH_3(CH_2)_{m-1}$ wherein m=12, 14, 16, 18, $R_2=R_3=R_4=$methyl and $A^-=Br^-$. The method of preparation is illustrated by the synthesis of the homologue with m=16 and $A^-=Br^-$.

Synthesis of hexadecyl trimethylphosphonium bromide

1-bromohexadecane (5mmol, 1.5ml) and trimethylphosphine (4mmol, 0.41ml) were dissolved in absolute ethanol (20ml) and refluxed under nitrogen in a sealed ampoule for 6 hours. The cooled reaction mixture was poured into 300 ml of diethyl ether and the resulting precipite was collected by vacuum filtration. The product was obtained as a white solid following 3 recrystallisations from absolute ethanol (0.91g, 60% yield).

Compounds of formula (IX).

A number of compounds of formula (IX) as defined above were synthesised by the alkylation of pyridine or its derivatives with the appropriate 1-bromoalkanes. The compounds prepared were those of formula (IX) $R_1=CH_3(CH_2)_{m-1}$ wherein m=12, 14, 16, 18, $R_2=R_3=R_4=H$ and $A^-=Br^-$ or Cl^-. The method of preparation is illustrated by the synthesis of the homologue with m=16 and $A^-=Br^-$.

Synthesis of hexadecylpyridinium bromide

1-bromohexadecane (5mmol, 1.5ml) was dissolved in 20ml dry pyridine and refluxed under nitrogen for 16 hours with rigorous exclusion of moisture. The excess pyridine was removed by short-path distillation and the remaining solid was dissolved in 10ml of hot dioxane. Dry acetone was added slowly until precipitation was observed. The precipitate was isolated by vacuum filtration and recrystallised twice from hot dioxane. The purified product was washed with 4x20 ml acetone and dried in a vacuum oven at 70°C. (1.88g, 90% yield).

Compounds of formula (X).

A number of compounds of formula (X) as defined above were synthesised by the alkylation of quinoline with the appropriate 1-bromoalkanes. The compounds prepared were those of formula (X) in which $R_1=CH_3(CH_2)_{m-1}$ wherein m=12, 14, 16, 18, $R_2=R_3=R_4=H$ and A^- $=Br^-$ or Cl^-.

Synthesis of hexadecylquinolinium bromide.

1-bromohexadecane (5mmol, 1.5ml) was dissolved in 40ml dry quinoline and refluxed under nitrogen for 36 hourse with rigorous exclusion of moisture. The excess quinoline was removed by short-path distillation and the remaining solid was dissolved in 20ml of hot dimethylformamide. Dry acetone was added slowly until precipitation was observed. The precipitate was isolated by vacuum filtration and recrystallized twice from hot dimethylformamide. The purified product was washed with 6x20 ml acetone and dried in a vacuum oven at 70°C. (1.31g, 61% yield).

EXAMPLE 11

A number of compounds of formula (XI) as defined above were prepared as illustrated below.

Synthesis of 3-O-hexadecyl-rac-glycerol (intermediate A)

Sodium hydride, 60% in oil (6.0g) was placed in a 500ml round-bottomed flask fitted with a reflux condenser, stirrer, nitrogen inlet, thermometer and a drying tube. Dry tetrahydrofuran (200ml) was then added, followed by rac-solketal(12.5 ml, 0.1mol) and 1-bromohexadecane (31.5ml, 0.15mol). The reaction mixture was refluxed with stirring under nitrogen at 60-67°C, for 3h monitoring the reaction by tlc (petroleum ether: ether 3:1). When all the starting material (R_f0.1) had been converted to the alkylated solketal, (R_f0.8) the solvent in the mixture was evaporated off from the filtrate to give a white solid and oil. Water (300ml) was added to dissolve the mixture, and the aqueous solution was extracted with ether (5x200ml). The organic phases were combined and concentrated on the rotary evaporator to give a yellow oil. Methanol/conc. HCl (9:1) (500ml) was added to

reflux for 2h, monitoring by tlc (100% ethyl acetate). The crude solid product (R_f0.7) formed was filtered off, and the filtrate obtained was concentrated to obtain more of the crude product. The whitish solid product was recrystallized twice from methanol, to give the pure product (A) as a white solid (18.50g. 58%).

Synthesis of 1-O-trityl-3-O-hexadecyl-rac-glycerol (intermediate B)

3-O-hexadecyl-rac-glycerol(A) (1.98g.6.25 mmol) and triphenylmethyl chloride (2.1g 7.5mmol) in dry pyridine (10ml) was refluxed at 100°C over a period of 10h, following the progress of reaction by tlc (petroleum ether:ether 3:1). The reaction mixture was allowed to cool to room temperature and was diluted with ether and ice water. The organic layer was separated off and the aqueous phase was extracted with ether (3x100ml). The organic layers were then combined and dried over $MgSO_4$, filtered and evaporation of the solvent yielded the crude product as a pale yellow oil. Purification by column chromatography on silica gel, eluted with petroleum ether:ether (3.1) gave the desired compound (A) as a white solid (2.79g, 80%).

Synthesis of 1-O-trityl-2-O-methyl-3-O-hexadecyl-rac-glycerol (intermediate C)

To a solution of 1-O-trityl-3-O-hexacadecyl-rac-glycerol (B) (2.8g, 0.01mol) in dry THF (50ml), a suspension of 60% sodium hydride in oil (0.22g, 6mmol) was added, and the resulting mixture was refluxed at 60°C for 0.5h. Next methyl iodide (0.71g. 0.01mol) was introduced into the mixture, and the reaction mixture was allowed to stir overnight at room temperature under nitrogen. Reduced pressure was

employed to remove the solvent to give a white residue, which was redissolved in ether (40ml). Any insoluble solid was filtered off and the colourless filtrate obtained was washed with sat. NaCl solution and dried with $MgSO_4$. Filtration and evaporation afforded the crude product as an yellow oil. This was purified by column chromatography over silica and petroleum ether:ether (7.3) as eluant to give the product (C) as a colourless oil (1.51g, 78%).

Synthesis of 3-O-hexadecyl-2-O-methyl-rac-glycerol (intermediate D)

1-O-trityl-2-O-methyl-3-O-hexadecyl-rac-glycerol (C) (2.42g, 4.22 mmol) was refluxed with a solution of glacial acetic acid (40ml) over a period of 5h, and the resulting mixture was allowed to cool to room temperature. The white solid was filtered off and dissolved in petroleum ether before being absorbed on ca. 1g of silica. Column chromatography (silica, petroleum ether: ether, 7:3) yielded the purified product (D) as a whitish waxy solid (0.9g, 65%).

Synthesis of 1-O-dichlorophosphate-2-O-methyl-3-O-hexadecyl-rac-glycerol (intermediate E)

Triethylamine (0.05g, 0.5mmol), was added to a solution of 3-O-hexadecyl-2-O-methyl-rac-glycerol (D) (0.082g, 0.25mmol) in CH_2Cl_2 (20ml) at room temperature, followed by the dropwise addition of a solution of phosphorus oxychloride (0.08g, 0.5mmol) in CH_2Cl_2 (2ml). The reaction mixture was allowed to stir at room temperature for about 2h under N_2, monitoring the progress of reaction by tlc (using ethyl acetate as eluant). On total conversion of the starting material (R_f0.55) to the phosphorodichloridate (E)(R_f0.9), the solvent and excess $POCl_3$ and NEt_3 were evaporated off at high vacuum to yield the crude

phosphorodichloridate (E) as a waxy solid. When used in further reactions compound (E) was dissolved in CH_2Cl_2 (20ml) and NEt_3 (0.05g, 0.5mmol).

<u>Synthesis of 1-O-(1',3'-diamino-2'-phosphacycloheptane-2'-oxide)-2-O-methyl-3-O-hexadecyl-rac-glycerol (target compound XI with $R_1=CH_3(CH_2)_{m'}-_1$ wherein m'=16, R_2=methyl, V=W=O, X=Y=NH, $R_3=R_4=H$, m=2 and n=1)</u>

1,4-butanediamine (44mg, 0.5mmol, 2eq.) in CH_2Cl_2 (2ml) was introduced into a solution of 1-O-dichlorophosphate-2-O-methyl-3-O-hexadecyl-rac-glycerol (E) and was allowed to stir at room temperature (20°C) under N_2 overnight. The crude product was obtained as a waxy whitish solid after solvent removal under vacuum, which on purification by chromatographic column (silica gel, 100% ethyl acetate) yielded the target compound as a waxy white solid (52mg, 45%).

<u>Synthesis of 1-O-(1',3'-diamino-2'-phosphacyclohexane-2'-oxide)-2-O-methyl-3-O-hexadecyl-rac-glycerol (target compound XI with $R_1=CH_3(CH_2)_{m'}-_1$ wherein m'=16, R_2=methyl, V=W=O, X=Y=NH, $R_3=R_4=H$, and m=n=1)</u>

1,3-propanediamine (37mg, 0.5mmol 2eq.) in CH_2Cl_2 (2ml) was introduced into a solution of 1-O-dichlorophosphate-2-O-methyl-3-O-hexadecyl-rac-glycerol (E) and was allowed to stir at room temperature (20°C) under N_2 overnight. The crude product was obtained as a waxy whitish solid after solvent removal under vacuum, which on purification by chromatographic column (silica gel. 100% ethyl acetate) yielded the target compound as a waxy white solid (52mg,48%).

1-O-(1'-N-methylamino-3'-methylamino-2'-phosphacyclohexane-2'-oxide)-2-O-methyl-3-O-hexadecyl-rac-glycerol (target compound XI with $R_1=CH_3(CH_2)_{m'-1}$ wherein m'=16, R_2=methyl, V=W=O, X=Y=NCH_3, $R_3=R_4$=H, and m=n=1)

N,N'-dimethyl-1,3-propanediamine (51mg, 0.50mmol, 2eq.) in CH_2Cl_2 (2ml) was introduced dropwise into a solution of 1-O-dichlorophosphate-2-O-methyl-3-O-hexadecyl-rac-glycerol (E) was stirred at 0°C under N_2 for 2h. Evaporation of the solvent and NEt_3 under vacuum gave the crude product as a waxy whitish solid, which after purification by flash chromatography column over silica gel (petroleum ether: ethyl acetate 1:1) yielded target compound as a waxy white solid (20mg, 15%).

Synthesis of 1-O-(1',3'-dioxa-2'-phosphacyclohexane-2'-oxide)-2-O-methyl-3-0-hexa-decyl-rac-glycerol (target compound XI with $R_1=CH_3(CH_2)_{m'-1}$ wherein m'=16, R_2=methyl, V=W=X=Y=O, $R_3=R_4$=H, and m=n=1)

1,3-propanediol (38mg, 0.5mmol, 2eq.) in CH_2Cl_2 (2ml) was introduced into a solution of 1-O-dichlorophosphate-2-0-methyl-3-0-hexadecyl-rac-glycerol (E) and was allowed to stir at 0°C under N_2 for 2h. The crude product (R_f0.16) was obtained as a waxy whitish solid after solvent removal under vacuum, which on purification by flash chromatography over silica gel eluting with petroleum ether: ethyl acetate (1:1), yielded the target compound as a waxy white solid (28.2mg, 26%).

Synthesis of 1-O-(1'-amino-3'-oxa-2'-phosphacyclohexane-2'-oxide)-2-O-methyl-3-0-hexa-decyl-rac-glycerol (target compound XI with $R_1=CH_3(CH_2)_{m'-1}$ wherein m'=16, R_2=methyl, V=W=X=O, Y=$R_3=R_4$=H, and m=n=1)

3-amino-propan-1-ol (37mg, 0.50mmol, 2eq.) in CH_2Cl_2 (2ml) introduced into a solution of 1-O-dichlorophosphate-2-O-methyl-3-O-hexadecyl-rac-glycerol (E) and was allowed to stir for 1h under N_2 at 0°C. The crude product was obtained after solvent removal as a yellowish solid, and was chromatographed over silica (petroleum ether: ethyl acetate 1:1) to give the target compound as a waxy white solid (47mg, 42%).

Synthesis of 1-O-(5',5'-dimethyl-1',3'-dioxa-2'-phosphacyclohexane-2'-oxide)-2-O-methyl-3-O-hexadecyl-rac-glycerol (target compound XI with $R_1=CH_3(CH_2)_{m'-1}$ wherein m'=16, R_2=methyl, V=W=X=Y=O, $R_3=R_4$=methyl and m=n=1)

2,2-dimethyl-1,3-propanediol (54mg, 0.50mmol, 2eq.) in CH_2Cl_2 (2ml) was introduced dropwise into a solution of 1-O-dichlorophosphate-2-O-methyl-3-O-hexadecyl-rac-glycerol (E) and was allowed to stir at 0°C under N_2 for 2h. The crude product was isolated after evaporation under vacuum as a waxy whitish solid, which on purification via chromatographic column (silica, petroleum ether: ethyl acetate 1:1) yielded the target compound as a waxy white solid (R_f 0.5) (60mg, 51%).

EXAMPLE 12

A number of further compounds used in the invention were synthesised.

A number of compounds of formula (XII) as defined above were synthesised by the procedures above for compounds of formula (XI). The appropriate n-alkanols were reacted with phosphorus oxychloride in the same manner as for the preparation of intermediate (E). The resulting alkyloxydichlorophosphate was then

reacted with the appropriate diol, diamine, aminoalcohol, dithiol or thioalcohol.

A number of compounds of formula (XIII) as defined above were synethesised by using 3-O-alkyl-2-O-methyl-rac-glycerol intermediates which were prepared according to the methods described above for compounds of formula (XI). The method is illustrated by the synthesis of a compound of formula (XIII) with $R_1=CH_3(CH_2)_{m-1}$ wherein m=16, R_2=methyl, V=W=O, X=O, Y=NH and $R_3=R_4$=ethyl.

Synthesis of 1-O-(bis(2,2,2-trichloroethyl)phosphate) -2-O-methyl-3-O-hexadecyl-rac-glycerol (intermediate F)

3-O-hexadecyl-2-O-methyl-rac-glycerol (intermediate D) (132mg, 0.4mmol) was dissolved in 5ml dry pyridine. To this solution was added bis(2,2,2-trichloroethyl)phosphorochloridate (228mg, 0.6mmol) dissolved in dry pyridine (1ml). The mixture was stirred under nitrogen for 2 hours. A further quantity (228mg, 0.6mmol) of bis (2,2,2-trichloroethyl)-phosphorochloridate dissolved in dry pyridine (1ml) was added to the reaction mixture. The mixture was stirred under nitrogen for a further 4 hours. Pyridine was removed under vacuum using short path distillation to afford a yellowish oil. Diethyl ether (10ml) was added and solids were removed by vacuum filatration. The ether solution was evaporated to dryness and gave a yellow oil. This was purified by column chromatography over silica using petroleum ether/diethyl ether (2:1) as eluant to afford intermediate (F) as a colourless oil (0.21g. 79% yield).

Synthesis of 1-O-(1'ethylamino, 1'-ethoxy phosphate)-2-O-methyl-3-O-hexadecyl-rac-glycerol

(target compound XIII with $R_1=CH_3(CH_2)_{m-1}$ wherein m=16, R_2=methyl, V=W=O, X=O, Y=NH and R_3 and R_4=ethyl).

1-O-(bis(2,2,2-trichloroethyl) phosphate1-2-O-methyl-3-O-hexadecyl-rac-glycerol (0.26g, 0.39mmol) was dissolved in dry ethylamine (20ml). Cesium fluoride (1.30g, 8.56mmol) was added and the reaction was stirred at 6°C for 4 days with rigorous exclusion of moisture. Excess ethylamine was evaporated and 20ml absolute ethanol was added. The mixture was allowed to stir for a further 3 days at room temperature (20°C). Excess ethanol was removed from under vacuum and the residue was purified by column chromatograph using silica gel and petroleum ether/ethyl acetate (1:1) as eluant. The target compound was obtained as a colourless oil (0.02g, 11% yield).

A number of compounds of formula (XIV) as defined above were synthesised by reacting 1-O-dichlorophosphate-2-O-methyl-1-O-alkyl-rac-glycerols (obtained as indicated for intermediate E) sequentially with primary n-alkanols and choline chloride.

A number of compounds of formula (XV) as defined above were synthesised by the acylation of 1-alkylamino sugars.

<u>**Synthesis of N-octyl-(1-octadecylamino-β-D-glucopyranoside (intermediate A)**</u>

N-octyl-β-glucopyranosylamine was synthesised as described by Attard G.S, Blackaby W.P. and Leach A.R. (1994), Chemistry and Physics of Lipids, vol 74, p83-91. N-octyl-β-glucopyranosylamine (1.5g, 15mmol) was dissolved in dry THF (40ml) and cooled to 0°C. Anhydrous sodium carbonate (0.546g. 1.5eq) was added. To this mixture was added, dropwise, octadecanoyl chloride (1.56g. 1.5eq.) dissolved in 10ml THF. Vigorous stirring was maintained

throughout the addition and subsequent reaction. The reaction mixture was allowed to warm to room temperature (20°C) overnight. Methanol (2x25ml) was added and the inorganic material removed by filtration. The filtrate was evaporated under reduced pressure to give a yellowish gummy solid. The crude material was purified by column chromatography on silica pre-eluted with 1% triethylamine in dichloromethane. The mixture was eluted with 5% methanol in dichloromethane to afford compound A as a colourless glassy solid (1.2g. 75% yield).

Synthesis of N-octyl-(1-octadecylamido-β-D-glucopyranosyl-6-O-ptoluenesulphate (intermediate B).

P-Toluenesulphonyl chloride (0.41g, 2.15mmol) was dissolved in 5ml dry pyridine. The solution was added dropwise with stirring to a solution of N-octyl-(1-octadecylamido)-β-D-glucopyranoside (A) (1.0g, 1.8mmol) in 20ml dry pyridine at 0°C. After warming to room temperature (20°C) over a period of 6 hours the solvent was removed under vacuum and residual pyridine was removed by co-evaporation with toluene. Diethyl ether (25ml) was added and the precipitate was removed by filtration. The filtrate was evaporated under vacuum to give a greenish oil. The oil was purified by column chromatography using silica and 4% methanol in chloroform as the eluant. The intermediate (B) was obtained as a colourless glassy solid (1.06g, 83% yield).

Synthesis of N-octyl(1-octadecylamido-β-D-(6-amino glucopyranoside(target compound XV with $R_1=NH_2$, $R_2=R_3=R_4=OH$, $R_5=CH_3(CH_2)_{m-1}$ wherein m=16 and $R_6=CH_3(CH_2)_{-1}$ wherein m=8

Ammonia gas was bubbled for 2 hours through 20ml of stirred and cooled (0°C) dry methanol. N-octyl-(1-octadecylamido)-β-D-glucopyranosyl-6-O-p-

toluenesulphate (200mg, 0.33mmol) was dissolved in this solution and the mixture was placed in a sealed tube and heated to 120°C for 16 hours. The tube was cooled to room temperature (20°C) and the solvent removed under vacuum. The gummy residue was dissolved in 10ml methanol and stirred with potassium hydroxide (200mg) for 2 hours. Solids were removed by filtration and filtrate was evaporated to dryness. The solid residue was purified by column chromatography using silica and 20% methanol in chloroform as the eluant. The target material was obtained as a colourless glassy solid (163mg. 88%).

CLAIMS:

1. Use of an amphiphilic compound in the manufacture of a medicament for the inhibition of phosphatidylcholine synthesis, said amphiphilic compound have the following properties:

  i the compound comprises a non-ionic, cationic or anionic hydrophilic head group and a hydrophobic tail group,

  ii the head group has a cross section A and the tail group has a cross section B such that the ratio B:A is less than 0.7:1,

  iii the tail group comprises a straight hydrocarbon chain having from 8 to 18 carbon atoms, and

  iv the amphiphilic compound has a membrane/water partition coefficient of more than 1×10^{-3}.

2. Use according to claim 1 wherein the ratio B:A is less than 0.5:1.

3. Use according to claim 1 wherein the amphilphilic compound is an oligoethyleneglycol monoalkyl ether of formula (I):

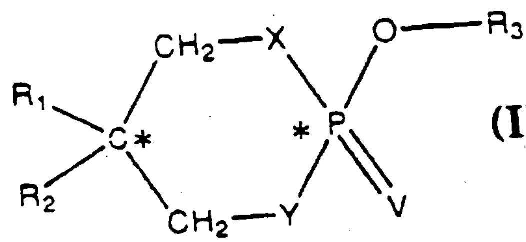
$$R_1\text{—}(OCH_2CH_2)_n\text{-}OH \quad \text{(I)}$$

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms, and

n is from 6 to 12.

4. Use according to claim 1 wherein the amphiphilic compound is an alkyl malonyl phosphoanhydride of formula (II):



(II)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing up to 5 carbon atoms,

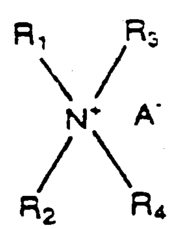
R_3 is hydrogen, a monovalent cation or a choline group,

X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH, and

V is oxygen or sulfur.

5. Use according to claim 1 wherein the amphiphilic compound is an alkylammonium compound of formula (III):



(III)

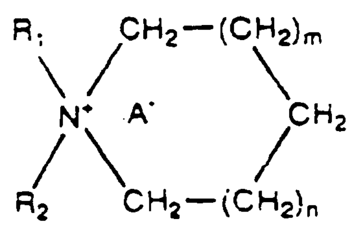
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2, R_3 and R_4, which may be identical or different, are each a methyl, ethyl or straight or branched propyl group, and

A^- is a pharmaceutically acceptable anion.

6. Use according to claim 1 wherein the amphiphilic compound is an alkylammonium compound of formula (IV):



(IV)

wherein:

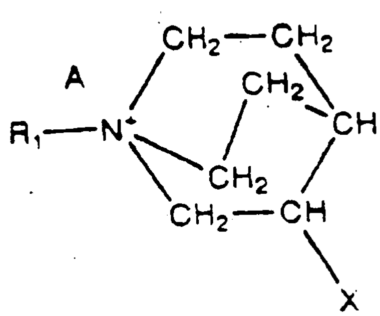
R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is methyl,

m and n are integers such that the ring contains from 5 to 8 ring atoms, and

A^- is a pharmaceutically acceptable anion.

7. Use according to claim 1 wherein the amphiphilic compound is an alkylammonium compound of formula (V):



(V)

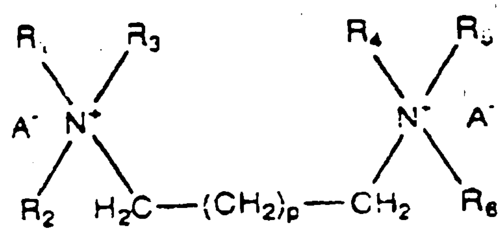
wherein:

R_1 is a straight hydrocarbon group containing from 8 to 18 carbon atoms,

X is hydrogen, and

A^- is a pharmaceutically acceptable anion.

8 Use according to claim 1 wherein the amphiphilic compound is an alkylammonium compound of formula (VI):



(VI)

wherein:

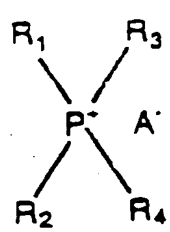
R_1, R_3, R_4 and R_5, which may be identical or different, are each methyl or ethyl,

R_2 and R_6, which may be identical or different, are each straight hydrocarbon groups containing from 10 to 18 carbon atoms

p is from 2 to 4, and

each A^-, which may be identical or different, is a pharmaceutically acceptable anion.

9. Use according to claim 1 wherein the amphiphilic compound is an alkylphosphonium compound of formula (VIII):



(VIII)

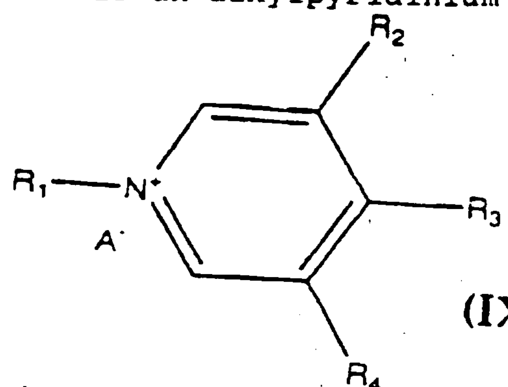
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2, R_3 and R_4, which may be the same or different, are each methyl, ethyl or straight or branched propyl groups, and

A^- is a pharmaceutically acceptable anion.

10. Use according to claim 1 wherein the amphiphilic compound is an alkylpyridinium compound of formula (IX):



(IX)

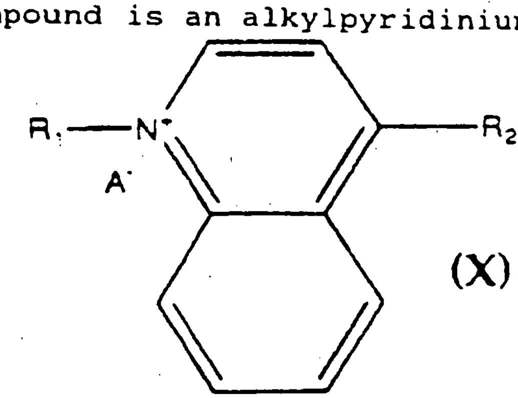
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2, R_3 and R_4 are each hydrogen, and

A^- is a pharmaceutically acceptable anion.

11. Use according to claim 1 wherein the amphiphilic compound is an alkylpyridinium compound of formula (X):



(X)

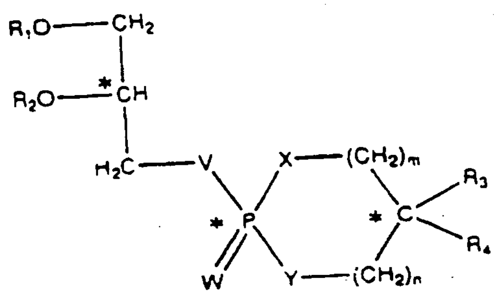
wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen, and

A^- is a pharmaceutically acceptable anion.

12. Use according to claim 1 wherein the amphiphilic compound is an alkyl trisubstituted phosphate of formula (XI):



(XI)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing not more than 5 carbon atoms,

R_3 and R_4, which may be identical or different, are each a straight or branched alkyl group containing not more than 5 carbon atoms,

m and n are integers such that the ring contains from 5 to 8 ring atoms,

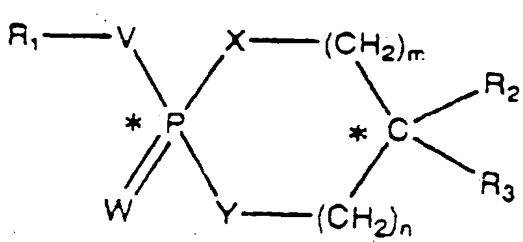
X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH

V is oxygen or sulfur, and

W is oxygen or sulfur.

13. Use according to claim 1 wherein the amphiphilic compound is an alkyl trisubstituted phosphate of formula (XII):



(XII)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is a straight or branched alkyl group containing not more than 5 carbon atoms,

R_3 is a straight or branched alkyl group containing not more than 5 carbon atoms,

m and n are integers such that the ring contains from 5 to 8 ring atoms

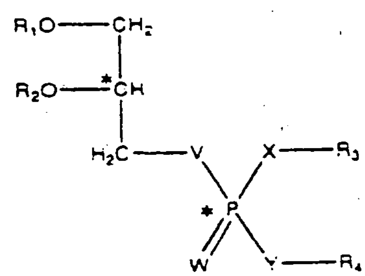
X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH

V is oxygen or sulfur, and

W is oxygen or sulfur.

14. Use according to claim 1 wherein the amphiphilic compound is a trisubstituted phosphate of formula (XIII):



(XIII)

wherein:

R_1 is a straight hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing not more than 5 carbon atoms,

R_3 and R_4, which may be identical or different, are each a straight or branched alkyl group containing not more than 5 carbon atoms,

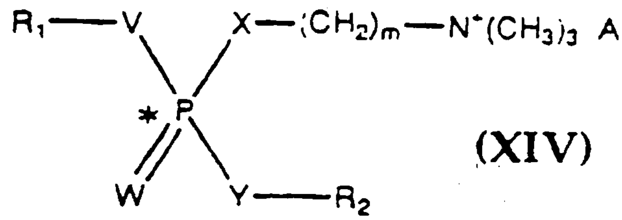
X is oxygen, sulfur or NH,

Y is oxygen, sulfur or NH

V is oxygen or sulfur, and

W is oxygen or sulfur.

15. Use according to claim 1 wherein the amphiphilic compound is a trisubstituted phosphate of formula (XIV):



(XIV)

wherein:

R_1 is a hydrocarbon group containing from 10 to 18 carbon atoms,

R_2 is hydrogen or a straight or branched alkyl group containing not more than 5 carbon atoms,

m is from 2 to 6

X is oxygen, sulfur or NH,

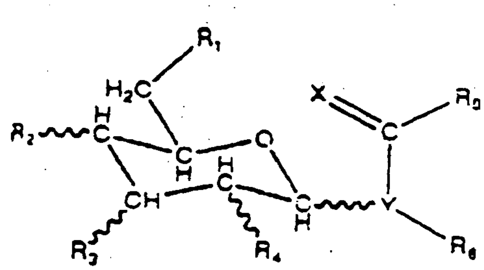
Y is oxygen, sulfur or NH

V is oxygen or sulfur,

W is oxygen or sulfur, and

A^- is a pharmaceutically acceptable anion.

16. Use according to claim 1 wherein the amphiphilic compound is a glycolipid analogue of formula (XV):



(XV)

wherein:

R_1 is an amine, trimethyl ammonium, triethyl ammonium or phosphate group,

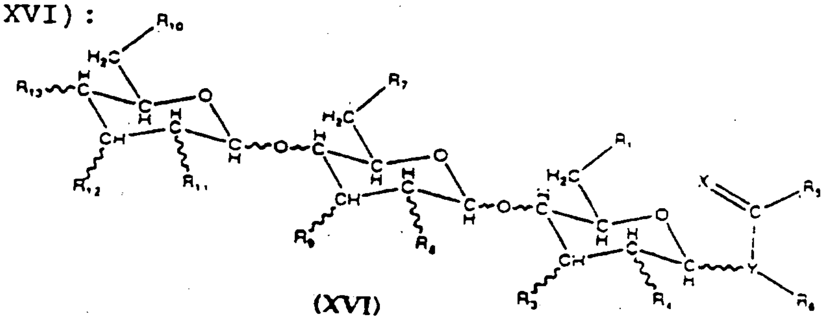
R_2 is a hydroxy or phosphate group,

R_3 is a hydroxy or phosphate group,

R_4 is hydrogen, and

one of R_5 and R_6 is methyl, ethyl or straight or branched propyl and the other is a straight hydrocarbon group containing from 8 to 18 carbon atoms.

17. Use according to claim 1 wherein the amphiphilic compound is a glycolipid analogue of formula (XVI):



(XVI)

wherein:

R_1, R_7 and R_{10}, which may be identical or different, is each an amine, trimethyl ammonium, triethyl ammonium or phosphate group,

R_{13} is a hydroxy or phosphate group,

R_3, R_9 and R_{12}, which may be identical or different, is each a hydroxy or phosphate group,

R_4, R_8 and R_{11} is each hydrogen, and

one of R_5 and R_6 is methyl, ethyl or straight or branched propyl and the other is a straight hydrocarbon group containing from 8 to 18 carbon atoms.

18. Use according to claim 1 for the treatment of a cancer.

19. An amphiphilic compound of formula (II), XI), (XII), (XIV), (XV) or (XVI) as defined in any one of claims 4, 12, 13, 15, 16 and 17.

20. An amphiphilic compound as defined in any one of claims 1 to 17 for use in a method of treatment of the human or animal body by therapy.

21. A pharmaceutical composition comprising an amphiphilic compound as defined in claim 1 and a pharmaceutically acceptable carrier or diluent.

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(57) Abstract

Use of an amphiphilic compound in the manufacture of a medicament for the inhibition of phosphatidylcholine synthesis, said amphiphilic compound have the following properties: i) the compound copmprises a non-ionic, cationic or anionic hydrophilic head group and a hydrophobic tail group; ii) the head group has a cross section A and the tail group has a cross section B such that the ratio B:A is less than 0.7:1; iii) the tail group comprises a straight hydrocarbon chain having from 8 to 18 carbon atoms; and iv) the amphiphilic compound has a membrane/water partition coefficient of more than 1×10^{-3}.

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MG	Madagascar	TJ	Tajikistan
MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
		TR	Turkey
ML	Mali	TT	Trinidad and Tobago
MN	Mongolia	UA	Ukraine
MR	Mauritania	UG	Uganda
MW	Malawi	US	United States of America
MX	Mexico	UZ	Uzbekistan
NE	Niger	VN	Viet Nam
NL	Netherlands	YU	Yugoslavia
NO	Norway	ZW	Zimbabwe
NZ	New Zealand		
PL	Poland		
PT	Portugal		
RO	Romania		
RU	Russian Federation		
SD	Sudan		
SE	Sweden		
SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02410

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/045 A61K31/66 A61K31/665 A61K31/675 A61K31/14
A61K31/55 A61K31/445 A61K31/44 A61K31/47 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 8543 Derwent Publications Ltd., London, GB; AN 85-266689 XP002046739 & JP 60 178 816 A (RIKAGAKU KENKYUSHO) , 12 September 1985 see abstract & DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US AN. CA: 104(12)95474k, TAKAHASHI, N. ET AL: "Anticancer pharmaceuticals containing surfactants as active ingredients" see abstract --- -/--	1-3,18, 20,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 1998	11.05.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MAIR J.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BECKERS, D. ET AL: "Molecular targets of Miltefosine" DRUGS OF TODAY, vol. 30, no. suppl. B, 1994, pages 5-12, XP002046737 see page 7, right-hand column, line 16-47 see page 8; figure 2 ---	1-3,18, 20,21
A	KELLEY, E.E. ET AL: "Unidirectional membrane uptake of the ether lipid antineoplastic agent Edelfosine by L1210 cells" BIOCHEMICAL PHARMACOLOGY, vol. 45, no. 2, 1993, pages 2435-2439, XP002046738 cited in the application see the whole document ---	1-3,18, 20,21
A	MATSUMOTO, Y. ET AL: "Specific hybrid liposomes composed of phosphatidylcholine and polyoxyethylenealkyl ether with markedly enhanced imnhibitory effects on the growth of tumor cells in vitro" BIOLOGICAL AND PHARMACEUTICAL BULLETIN, vol. 18, no. 10, October 1995, pages 1456-8, XP000537683 see the whole document ---	1-3,18, 20,21
X	WIEDER,T. ET AL: "The effect of two synthetic phospholipids on cell proliferation and phosphatidylcholine biosynthesis in Madin-Darby canine kidney cells" LIPIDS, vol. 30, no. 5, 1995, pages 389-393, XP002061923	1,2,15, 18-21
Y	see the whole document especially page 389, scheme 1 ---	3,6,10, 11,13
X	GEILEN, C.C. ET AL: "Phosphatidylcholine biosynthesis as a target for phospholipid analogues" ADV.EXP.MED.BIOL. (PLATELET-ACTIVATING FACTOR AND RELATED LIPID MEDIATORS 2), vol. 416, 1996, pages 333-336, XP002061924	1,2,15, 18-21
Y	see the whole document ---	3,6,10, 11,13

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOGGS, KEVIN P. ET AL: "Lysophosphatidylcholine and 1-O-Octadecyl-2-O-Methyl-rac-Glycero-3-Phosphocholine inhibit the CDP-Choline pathway of phosphatidylcholine synthesis at the CTP:Phosphocholine Cytidylyltransferase step" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 13, 31 March 1995, pages 7757-7764, XP002061925 see the whole document ---	1-3,6, 10,11, 13,15, 18-21
Y	PLANTAVID M ET AL: "CATIONIC AMPHIPHILIC DRUGS AS A POTENTIAL TOOL FOR MODIFYING PHOSPHOLIPIDS OF TUMOR CELLS. AN IN VITRO STUDY OF CHLORPROMAZINE EFFECTS ON KREBS II ASCITES CELLS" BIOCHEM PHARMACOL;30(4):293-297 1981, XP002061926 see the whole document ---	1-3,6, 10,11, 13,15, 18-21
X	MACKENZIE, A.N.: "The synthesis of novel phospholipids (HIV-1, immune deficiency, antineoplastic agents)." DISSERTATION ABSTRACTS INTERNATIONAL, vol. 57, no. 3-C, 1995, page 947 XP002061927 see abstract ---	1,2,13, 15,18-21
X	ATTARD, G.S. ET AL: "Phase behaviour of novel phospholipid comounds" CHEMISTRY AND PHYSICS OF LIPIDS, vol. 76, no. 1, 1995, pages 41-48, XP002061928 see the whole document ---	1,2,13, 15,21
X	EP 0 108 565 A (TAKEDA CHEMICAL INDUSTRIES) 16 May 1984 see the whole document ---	1,2,13, 15,21
X	DICK D L ET AL: "PHYSICOCHEMICAL BEHAVIOR OF CYTOTOXIC ETHER LIPIDS" BIOCHEMISTRY, 31 (35). 1992. 8252-8257., XP002061929 see the whole document ---	1,2,13, 15,21
X	COY, E.A. ET AL: "Antiproliferative effects of amphiphilic molecules" INTERNATIONAL JOURNAL OF IMMUNOPHARMACOLOGY, vol. 12, no. 8, 1990, pages 871-881, XP002061930	1,2,10, 18,20,21
A	see page 878; table 8 ---	11

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOE, D.W. ET AL: "Interaction of multidrug-resistant Chinese hamster ovary cells with amphiphiles" BRITISH JOURNAL OF CANCER, vol. 68, no. 2, 1993, pages 342-351, XP002061931	1,2,10, 18,20,21
A	see the whole document	11
X	DELLINGER, M. ET AL: "Structural requirements of simple organic cations for recognition by multidrug resistant cells" CANCER RESEARCH, vol. 52, no. 22, 15 November 1992, pages 6385-6389, XP002061932	1,2,10, 18,20,21
A	see the whole document	11
A	ROTENBERG, S.A. ET AL: "Inhibition of rodent protein kinase C by the anticarcinoma agent dequalinium" CANCER RESEARCH, vol. 50, no. 3, 1990, pages 677-685, XP002061933 see the whole document	1-3,10, 11,18, 20,21
Y	DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US Abstract number: 114:177982, XP002061940 see abstract & NISHISAKI, H. ET AL: "Inhibitory effects of antitumor drugs on phosphatidylcholine synthesis and its reversal by teprenone in isolated guinea pig gastric glands" IGAKU NO AYUMI, vol. 155, no. 10, 1990, pages 669-670,	1-3,6, 10,11, 13,15, 18-21
A	RAYNOR R.L. ET AL: "Membrane interactions of amphiphilic polypeptides mastoparan, melittin, polymyxin B, and cardiotoxin" J. BIOL. CHEM., 1991, 266/5 (2753-2758), USA, XP002061934 see the whole document	1-3,6, 10,11, 13,15, 18-21
A	BOURNE RK: "CHEMICAL SYNTHESIS AND ANTITUMOR ACTIVITY OF PHOSPHOGLYCERIDE MUSTARDS." DISS ABSTR INT (SCI);37(5):2261-B-2262-B 1976, XP002061935 see abstract	1-3,6, 10,11, 13,15, 18-21

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02410

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HILLYARD, L.A. ET AL: "Membrane proliferation and phosphatidylcholine synthesis in normal, preneoplastic and neoplastic mammary gland tissues in C3H mice" CANCER RESEARCH, vol. 32, no. 12, 1972, pages 2834-2842, XP002061936 see the whole document	1-3,6, 10,11, 13,15, 18-21
X	WOOD, S.J. ET AL: "Selective inhibition of A.beta fibril formation" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 8, 23 February 1996, pages 4086-4092, XP002061937 see the whole document	1,2, 19-21
X	READ, GEORGE W. ET AL: "Competitive inhibition of 48/80-induced histamine release by benzalkonium chloride and its analogs and the polyamine receptor in mast cells" THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 222, no. 3, 1982, pages 652-7, XP002061938 see the whole document	20,21
X	KUCZERA, J. ET AL: "Effects of some cyclic elements containing amphiphilic compounds on stability and transport properties of model lecithin membranes" GENERAL PHYSIOLOGY AND BIOPHYSICS, vol. 6, no. 6, December 1987, pages 645-654, XP002061939	20,21
A	see the whole document	1,2,6,18

INTERNATIONAL SEARCH REPORT

International application No. PCT/GB 97/02410

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1&2 PARTIALLY
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see further information PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see further informatino PCT/ISA/210

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1,2, 18-21, partially 3,6,10,11,13,15 completely

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ GB 97/02410

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims 1,2,18,20 and 21 (partially) and 3. Use of an oligoethyleneglycol monoalkyl ether compound of formula (I) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

2. Claims 1,2,18,19,20 and 21 (partially) and 4. Use of an alkyl malonyl phosphoanhydride compound of formula (II) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers and novel compounds of formula (II).

3. Claims 1,2,18,20 and 21 (partially) and 5. Use of an alkylammonium compound of formula (III) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

4. Claims 1,2,18,20 and 21 (partially) and 6. Use of an alkylammonium compound of formula (IV) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

5. Claims 1,2,18,20 and 21 (partially) and 7. Use of an alkylammonium compound of formula (V) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

6. Claims 1,2,18,20 and 21 (partially) and 8. Use of an alkylammonium compound of formula (VI) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

7. Claims 1,2,18,20 and 21 (partially) and 9. Use of an alkylphosphonium compound of formula (VIII) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

8. Claims 1,2,18,20 and 21 (partially) and 10 and 11. Use of an alkylpyridinium compound of formula (IX) and (X) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

9. Claims 1,2,18,19,20 and 21 (partially) and 12. Use of an alkyl trisubstituted phosphate compound of formula (XI) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers and novel compounds of formula (XI).

10. Claims 1,2,18,19,20 and 21 (partially) and 13. Use of an alkyl trisubstituted phosphate compound of formula (XII) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers and novel compounds of formula (XII).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

11. Claims 1,2,18,20 and 21 (partially) and 14. Use of a trisubstituted phosphate compound of formula (XIII) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers.

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12. Claims 1,2,18,20 and 21 (partially) and 15. Use of a trisubstituted phosphate compound of formula (XIV) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers and novel compounds of formula (XIV).

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13. Claims 1,2,18,20 and 21 (partially) and 16. Use of a glycolipid analogue of formula (XV) and (XVI) for the inhibition of phosphatidyl choline synthesis and the treatment of cancers and novel compounds of formula (XV) and (XVI).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. onal Application No
PCT/GB 97/02410

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 108565 A	16-05-84	JP 59084824 A US 4935520 A	16-05-84 19-06-90

Form PCT/ISA/210 (patent family annex) (July 1992)